



**UNIVERSITI PUTRA MALAYSIA**

**ERYTHROCYTE OSMOTIC FRAGILITY IN SHEEP AFFECTED  
BY *EPERYTHROZON OVIS***

**MARINA BT. HASSAN**

**FPV 2002 2**

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2002**

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**MARINA BT. HASSAN**

**Universiti Putra Malaysia, In Filfilment of the Requirement  
for the Degree of Master of Veterinary Science**

**January 2002**

*Specially dedicated to.....*

**Ayah, Ma, Hafiz & Syahidah Hana**

*For your love , support & trust*

**Man, Jah & Adriana, Nanie & Mi,  
Adik A & G**

*Thanks for everything.....*

Abstract of thesis presented to the Senate of Universiti Putra  
Malaysia in fulfilment of the requirement for the degree of Master  
of Veterinary Science

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**January 2002**

**Chairman : Associate Professor Dr. Che Teh Fatimah  
Nachiar Iskandar**

**Faculty : Veterinary Medicine**

A cross-sectional study design was conducted to detect the eperythrozoonosis in Malaysia environment. Twenty-five sheep were randomly selected from which sixty-two blood samples were collected.

Ethylene diamine tetracetic acid (EDTA), flouride and plain vacutainer tubes (Becton Dickinson, USA) were used to collect blood from the jugular vein of sheep. The fresh blood from EDTA was used to prepare thin blood smears, stained with Wright's

stain and examined microscopically under x40 and x100 magnification, to detect the presence of haemoparasites. The *E. ovis* scoring was determined as 0, positive 1, 2 and 3 to observe the severity of parasitaemia. The fresh blood was also used to determine haematology parameter tests such as white blood cell (WBC), red blood cell (RBC), packed cell volume (PCV), haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), icterus index and plasma protein determination. Serum and plasma were also taken for clinical biochemistry parameter tests such as the concentrations of glucose, cholesterol, triglyceride, serum gamma glutamyl transferase ( $\gamma$ GT) and blood urea nitrogen (BUN). Plasma from the flouride tubes was used for the glucose test. All the data obtained from the sheep were analysed using a correlation test and ANOVA test.

The study showed that WBC, RBC, haemoglobin, PCV, MCV, MCHC, icterus index and plasma protein were correlated with the different parasitaemia score. There was no significant difference ( $p < 0.05$ ) in the haematology parameters of animals with different parasitaemia scores. However, a correlation analysis showed a correlation between the haematology parameters and parasitaemia at different times of infection. The WBC, RBC,

haemoglobin, PCV and plasma protein parameters were negatively correlated with the parasitaemia after eight days of infection. The MCV was positively and MCHC was negatively correlated with parasitaemia after four days of infection.

There was no significant difference in the concentrations of glucose, cholesterol,  $\gamma$ GT and BUN parameters of animals with different parasitaemia scores. But, triglyceride was significantly difference with different parasitaemia scores. However, a correlation analysis showed correlation between the clinical biochemistry parameters and parasitaemia at different times of infection. Glucose levels were negatively correlated with the parasitaemia after four days of infection. Cholesterol levels were negatively correlated with the parasitaemia after two days of infection.  $\gamma$ GT and BUN were positively correlated after four days of infection. Triglyceride were negatively correlated after 10 days of infection. The clinical biochemistry parameters showed that the *E. ovis* activity on the RBC membrane did not affect the parameters studied.

There was no significant difference in the erythrocyte osmotic fragility (EOF) with different parasitaemia score. The mean EOF graph showed the same trend in all parasitaemia score. The

haematology parameters such as RBC, haemoglobin, PCV, MCV and icterus index were positively correlated whereas the MCHC and plasma protein were negatively correlated with the EOF. Glucose, triglyceride and  $\gamma$ GT levels were positively correlated whereas cholesterol and BUN levels were negatively correlated with the EOF.

The histopathology changes in the spleen, liver and kidney were mild to moderate. It showed that extravascular haemolysis occurred in the *E. ovis* infection.

In conclusion, the *E. ovis* detected in Malaysia did not greatly affect the haematology parameters, clinical biochemistry parameters and EOF. However, the effects of *E. ovis* infection were detected in spleen, liver and kidney. In conclusion, the *E. ovis* found in Malaysia was mildly pathogenic.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra  
Malaysia sebagai memenuhi keperluan untuk Ijazah Master  
Sains Veterinar

**KERAPUHAN OSMOSIS ERITROSIT PADA BEBIRI YANG  
DIJANGKITI *EPERYTHROZON* OVIS**

Oleh

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Pengajian jenis 'cross-sectional' telah dijalankan untuk mengesan penyakit eperythrozoonosis di Malaysia. Dua puluh lima ekor bebiri dipilih secara rawak daripadanya enam puluh dua contoh darah telah diambil.

Tiub Ethylene diamine tetracetic acid (EDTA), florida dan tiub kosong (Becton Dickinson, USA) telah digunakan untuk mengumpul darah daripada salur darah jugular pada bebiri. Darah segar daripada EDTA digunakan untuk penyediaan

calitan nipis darah, diwarnakan dengan pewarna Wright dan diperiksa dibawah mikroskop menggunakan kuasa x40 dan x100, untuk mengesan kehadiran parasit darah. Skor *E. ovis* dinyatakan sebagai 0, positif 1,2 dan 3 untuk mengkaji 'severity' kitaran parasit dalam darah. Darah segar juga digunakan untuk menguji parameter hematologi seperti sel darah putih (WBC), sel darah merah (WBC), hemoglobin, isipadu sel padat (PCV), min isipadu korpusel (MCV), min kepekatan hemoglobin korpusel (MCHC), indek ikterus dan protin plasma. Serum dan plasma diambil juga untuk menguji parameter biokimia klinikal seperti konsentrasi glukosa, kolesterol, triglyserida, serum gamma glutamyl transferase ( $\gamma$ GT) dan urea nitrogen darah (BUN). Plasma dalam tiub florida digunakan untuk pengujian glukosa. Semua data diperolehi di analisis dengan menggunakan pengujian korelasi dan ANOVA.

Pengajian ini menunjukkan WBC, RBC, hemoglobin, PCV, MCV, MCHC, indek ikterus dan protein plasma mempunyai korelasi dengan skor parasit yang berbeza. Tiada perubahan signifikan ( $p < 0.05$ ) pada parameter hematologi bebiri itu dengan skor parasit. Walaubagaimana pun, analisis korelasi menunjukkan ada korelasi antara parameter hematologi dan parasit dalam darah pada masa jangkitan yang berbeza. Parameter WBC, RBC,

hemoglobin, PCV dan protin plasma adalah korelasi negatif dengan parasit selepas lapan hari jangkitan. MCV adalah positif dan MCHC pula korelasi negatif selepas empat hari jangkitan.

Tiada perubahan signifikan dalam konsentration glukosa, kolesterol,  $\gamma$ GT dan BUN parameter bebiri dengan skor parasit yang berbeza. Tetapi, trigliserida perubahan signifikan dengan skor parasit yang berbeza. Walaubagaimana pun, analisis korelasi menunjukkan ada korelasi antara parameter biokimia klinikal dan parasit pada masa jangkitan yang berbeza. Tahap glukosa adalah korelasi negatif dengan parasit selepas empat hari jangkitan. Tahap kolesterol adalah korelasi negatif dengan parasit selepas dua hari jangkitan.  $\gamma$ GT dan BUN korelasi positif selepas empat hari jangkitan. Trigliserida korelasi negatif selepas sepuluh hari jangkitan. Parameter biokimia klinikal menunjukkan aktiviti *E. ovis* atas membran RBC tidak ada kesan pada parameter yang dikaji.

Tiada perubahan signifikan pada kerapuhan osmosis eritrosit (EOF) dengan skor parasit yang berbeza. Graf min EOF menunjukkan trend yang sama pada semua skor parasit. Parameter hematologi seperti RBC, hemoglobin, PCV, MCV dan indeks ikterus adalah korelasi positif dimana MCHC dan protein

plasma adalah korelasi negatif dengan EOF. Tahap glukosa, trigliserida dan  $\gamma$ GT adalah korelasi positif di mana kolesterol dan BUN adalah korelasi negatif dengan EOF.

Perubahan histopathologi pada limpa, hati dan ginjal adalah 'mild' ke 'moderate'. Ini menunjukkan hemolisis 'extravascular' berlaku dalam jangkitan *E. ovis*.

Pada kesimpulannya, *E. ovis* yang dikesan di Malaysia tidak memberi kesan besar pada parameter hematologi, parameter biokimia klinikal dan EOF. Walaubagaimana pun, kesan jangkitan *E. ovis* telah dikesan pada limpa, hati dan ginjal. Pada kesimpulannya, *E. ovis* yang ditemui di Malaysia adalah kurang patogenik.

## **ACKNOWLEDGEMENTS**

I would like to express my deepest and sincere gratitude and appreciation to my supervisor, Associate Professor Dr. Che Teh Fatimah Nachiar Iskandar and the members of the supervisory committee, Assoc. Prof. Dr. Rasedee Abdullah, Assoc. Prof. Dr. Mohd. Hair Bejo and Dr. Ungku Chulan Ungku Mohsin for their invaluable guidance, advice, supervision, kindness and support throughout the course of this study.

To my family, Hafiz and my daughter, Syahidah, thank you for the understanding, support and trust.

Finally to all my friends, Dr. Siti Norhayati, Dr. Siti Nor, Dr. Zamirah, Dr. Zurina, Dr. Wan Mastura, Dr. Mariah, Dr. Goh Yong Meng, Cik Azilah and Pn. Maizatul, my deepest appreciation for their help and support during the course of the project. To the Clinical Biochemistry laboratory staff, En. Helmi and En. Abdullah, I extend my thanks for their help throughout the course of this study.

I certify that an Examination Committee met on 18<sup>th</sup> January 2002 to conduct the final examination of Marina binti Hassan on her Master of Veterinary Science thesis entitled “Erythrocyte Osmotic Fragility in Sheep Affected by *Eperythrozoon ovis*” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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Marina Hassan

Date : 6.5.2002



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